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## INTRODUCTION

AIB1 (Amplified In Breast cancer 1) is a member of the p160 family of steroid receptor coactivators. AIB1 is located on chromosome 20q and its gene is amplified in 5-10% of primary breast cancers [1]. AIB1 mRNA is overexpressed in 60% of breast tumors [2] and its increased protein levels have been correlated with invasive ductal carcinoma [3]. AIB1 has been positively correlated with tumor size, p53 status and Her2/neu expression [4, 5]. High expression of AIB1 in breast tumors, together with high Her2/neu expression in patients that received tamoxifen therapy, had the worst disease free outcome compared to all other patients [6]. AIB1 has also been demonstrated to be a down stream target for phosphorylation by MAP kinase [7] and IkappaB kinase [8]. These data suggest that AIB1 may have a wide-ranging effect on tumor growth that is independent of steroid receptor function.

## BODY

The research accomplishments described in this report cover **Task 1** in the approved **Statement of Work** in the original grant application, DAMD17-00-1-0257. The goal of Task 1 was to examine the ability of AIB1 and the  $\Delta$ exon3 AIB1 isoform to potentiate growth factor signaling, such as EGF and IGF-1, in contributing to hormone independent growth of breast cancer. The first part of **Task 1** was to screen EGFR positive breast cancer cell lines to determine a model system to address the tasks of the approved grant. The MCF-7 breast cancer cell line was chosen as the model cell line to address the goals of **Task 1** for the following reasons: positive for both AIB1 and the  $\Delta$ exon3 AIB1 isoform, responds to growth stimulation by IGF-1 [9-11] and EGF [11-13],

and is positive for the IGF-1 [14] and EGF receptors [15]. The MCF-7 breast cancer cell line expresses more AIB1 than other breast cancer cell lines (both ER positive and ER negative) tested in our lab. MCF-7 cells were used to determine if EGF could induce the expression of AIB1 mRNA (Figure 1). Treatments with EGF were performed with serum starved MCF-7 cells and cytoplasmic RNA was collected after 6, 24 and 48 hours. The changes in AIB1 message were analyzed by Northern blot and quantitated using a densitometer. Even after 48 hours of EGF treatment, AIB1 mRNA did not increase compared to the negative control (IMEM) (Figure 1). The role of AIB1 in other breast cell lines, such as the MDA-231 and MDA-468 cell lines, are currently under investigation. MDA-231 cell line, for example, is a good cell line to examine the role of AIB1 and  $\Delta$ exon3 AIB1 in contributing to hormone independent growth because it is ER negative and IGF-1R and EGFR positive.

To address the second part of **Task 1**, the FGF-BP (fibroblast growth factor binding protein) promoter construct was transiently transfected with either full length AIB1 or  $\Delta$ exon3 AIB1 into ME-180 squamous carcinoma cells. Our lab has published several papers that demonstrate the responsiveness of the FGF-BP promoter in ME-180 to multiple mitogenic stimulation, including EGF [16-19]. The AP-1, ETS, and C/EBP sites were shown to be necessary for the EGF responsiveness of the promoter. These sites are contained in the first 118 base pairs (-118/+62) of the transcriptional start site. Deletions of the ETS and C/EBP sites or mutations in the AP-1 site within the -118/+62 were constructed previously [16, 19]. The promoter studies were done initially in ME-180 cells due to their well-defined responsiveness to EGF and induction of the FGF-BP promoter. Both AIB1 and  $\Delta$ exon3 AIB1 were able to coactivate the FGF-BP promoter in

the presence of EGF.  $\Delta$ exon3 AIB1 was able to induce the promoter up to 20 fold higher than full length AIB1 (Figure 2a). When AIB1 or  $\Delta$ exon3 AIB1 were cotransfected with mutant AP-1, deleted ETS or C/EBP plasmids into ME180 cells in the presence of EGF, no changes in the induction of the FGF-BP reporter plasmid were observed (Figure 2b-d). This indicated that AP-1, ETS or C/EBP were not involved or necessary for the coactivation of AIB1 or  $\Delta$ exon3 AIB1.

The second part of **Task 1** involved confirming the potentiating effects of AIB1 and  $\Delta$ exon3 AIB1 on an endogenous cellular gene. Classical ER responsive genes, such as EBAG9 or estrogen receptor binding fragment-associated gene 9) [20] and cathepsin D mRNA levels [21] were used to determine if transiently transfected AIB1 or  $\Delta$ exon3 AIB1 could increase their expression in MCF-7 cells as measured by real time PCR. Figure 3 demonstrates that  $\Delta$ exon3 AIB1 is more effective in increasing the expression of endogenous EBAG9 and cathepsin D genes compared to full length AIB1.

Status of Task 2: Experiments described in the approved grant application are still ongoing.

Status of Task 3: Experiments will be performed as described in the approved grant application on completion of **Task 1** and **2**.

Abbreviations:

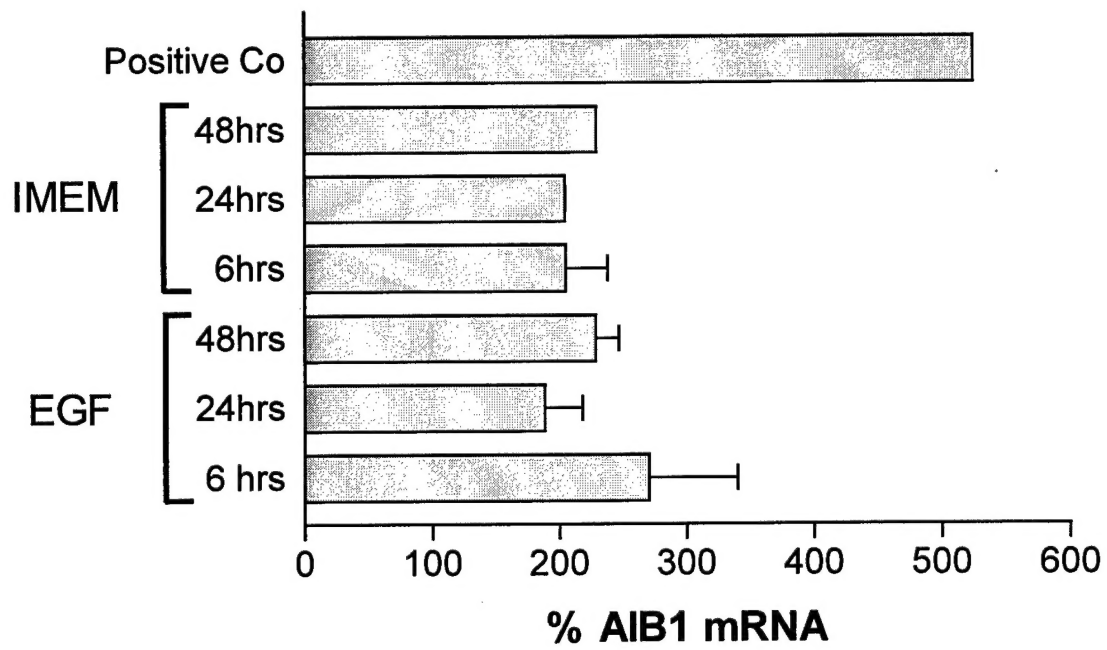
ETS – E26 transformation specific.

C/EBP – CCAAT enhancer-binding protein

AP-1 – activator protein 1

**Figure 1.** The potentiating effects of AIB1 or the  $\Delta$ exon3 AIB1 isoform on an EGF responsive promoter does not seem to be dependent on AP-1, ETS or C/EBP sites. ME180 squamous carcinoma cells were transiently transfected with control vector (Co; 3 $\mu$ g), pCDNA3-AIB1 (3 $\mu$ g) or pCR3-  $\Delta$ exon3 AIB1 and -118/+62, delta AP-1/-118/+62, delta Ets/-118/+62 or delta C/EBP/-118/+62 FBF-BP promoter-luciferase reporter plasmid (1 $\mu$ g) using LipofectAMINE™ (Invitrogen). Cells were treated with EGF (5ng/mL) for 18-24 hours, harvested and luciferase activity was measured. 0.1ng of pCMV-renilla vector was also transfected in all samples and renilla activity was used as a transfection control. Relative light unit (RLU) represents luciferase activity corrected with renilla activity.

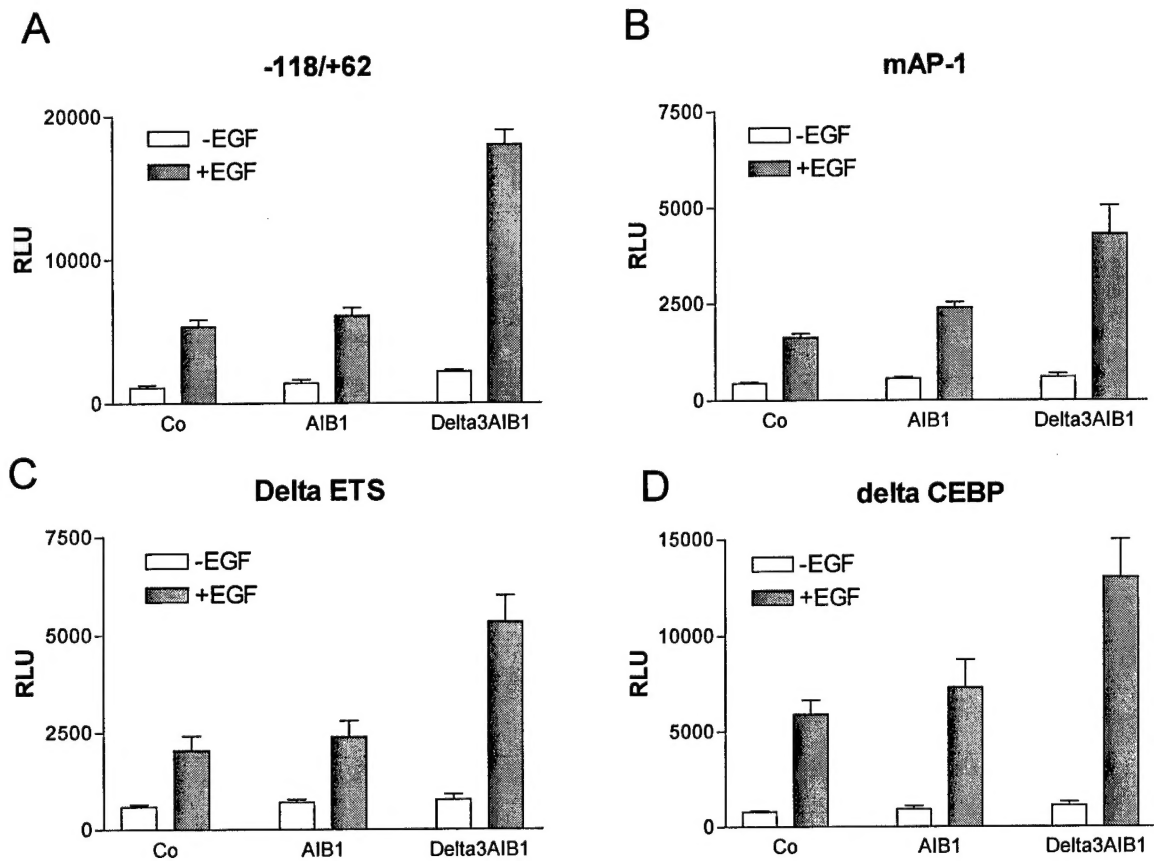
# Figure 1





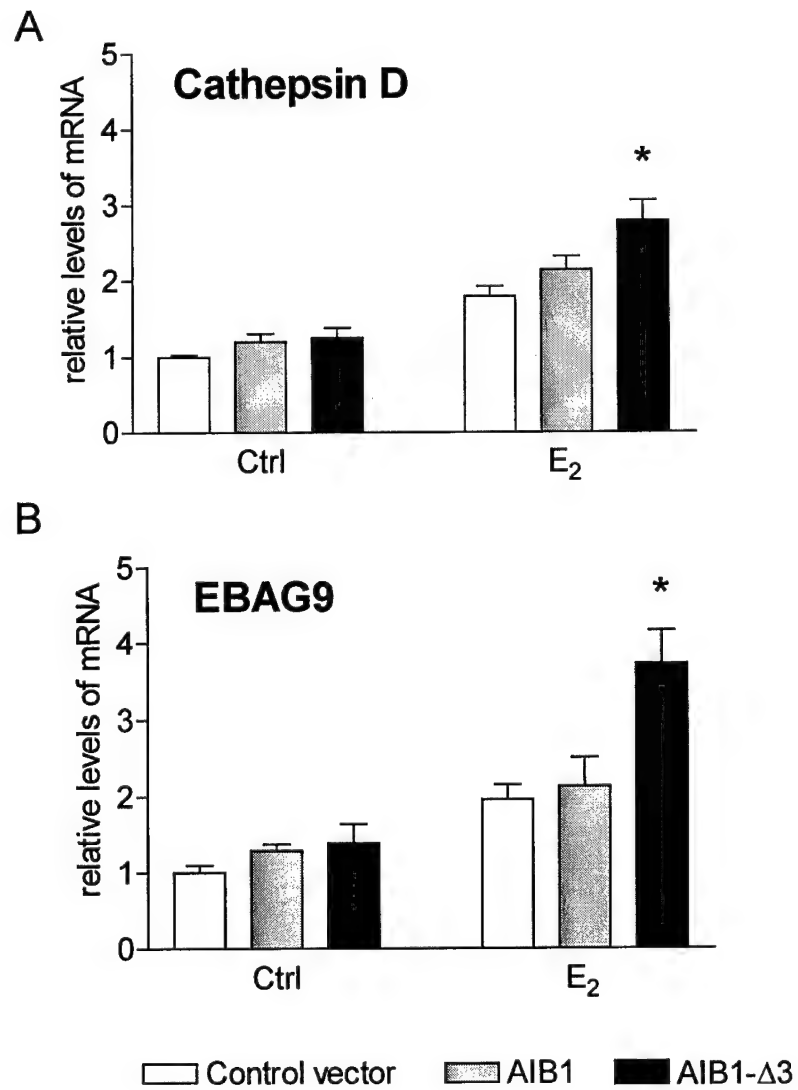
**Figure 2.** AIB1 message levels are not altered in MCF-7 cells after up to 48 hours of treatment with EGF. MCF-7 cells were serum starved overnight. Cells were treated with 100ng/ml of EGF for 6, 24, or 48 hours. Cytoplasmic RNA was harvested after treatment and 15µg of RNA was run on a 1% agarose/formaldehyde gel. A 0.75kb EcorRI fragment from AIB1 cDNA was used as a probe to hybridize the northern blot. AIB1 levels were quantitated using a densitometer and loading was corrected with GAPDH. Samples were run in duplicate. IMEM, improved modified eagles media; EGF, epidermal growth factor; Positive Co, positive control is retinoic acid treatment for 24 hours.

# Figure 2



**Figure 3.** Effects of AIB1 and AIB1- $\Delta$ 3 on the expression of CATD and EBAG9 in MCF-7 stimulated with estrogen. Cells were plated at 25% confluence in six-well plates and were cultured for IMEM supplemented with 5% FBS that had been treated with dextran-coated charcoal. Cells were then transfected with 3 $\mu$ g of either the empty pcDNA3 vector, pcDNA3-AIB1, or pcDNA3-AIB1- $\Delta$ 3 together with an expression vector for human ER- $\alpha$  (100ng) by using LipofectAMINE™ 2000 (Invitrogen) according to the manufacturer's instructions. 5 hours after transfection the media was replaced with IMEM containing 5% charcoal stripped FBS and 1nM ICI 182780. The next day cells were treated with either ethanol alone or 100nM 17 $\beta$ -estradiol for 2 h before RNA was harvested and CATD (panel A) and EBAG 9 (panel B) expression were measured by real time RT-PCR. mRNA levels were normalized by GAPDH mRNA expression, and the normalized values were then expressed relative to that of control cells transfected with pcDNA3 and treated with ethanol alone. Data represent mean  $\pm$  S.E. of values from two independent experiments, each performed in duplicate. \*,  $p < 0.05$  *versus* the corresponding value for control cells.

# Figure 3



## KEY RESEARCH ACCOMPLISHMENTS

- EGF was determined not to regulate endogenous AIB1 message levels as detected by Northern blot analysis.
- The ability of AIB1 and the splice variant,  $\Delta$ exon3 AIB1 to co-activate EGF signaling is independent of AP-1, ETS or C/EBP promoter elements.
- The higher co-activating effects  $\Delta$ exon3 AIB1, compared to full-length AIB1, were confirmed on the endogenous genes EBAG9 and Cathepsin D in MCF-7 cells.

## REPORTABLE OUTCOMES

### Abstracts:

*Oh, A.*, List, H.J., Reiter, R., Wellstein, A., and Riegel, A.T. The Nuclear Receptor Coactivator AIB1 has a rate-limiting role in IGF-1 dependent Growth In MCF-7 Breast Cancer Cells. Fifth Annual Lombardi Research Fair (2003). 1<sup>st</sup> Place. Pre-doctoral Division.

*Oh, A.*, List, H.J., Mani, A., Bowden E.T., Reiter, R., Wellstein, A., and Riegel, A.T. The Nuclear Receptor Coactivator AIB1 has a rate-limiting role in IGF-1 dependent Growth In MCF-7 Breast Cancer Cells. Abstract No. 4967. American Association of Cancer Research, Toronto, Canada (2003).

Reiter, R., *Oh, A.*, Lauritsen, K.J., Wellstein, A. and Riegel, A.T. An isoform of AIB1 (AIB1- $\Delta$ 3) increases the estrogenic activity of both agonists and partial agonists of the

estrogen receptor. Abstract No. 4908. American Association of Cancer Research, Toronto, Canada (2003).

## CONCLUSIONS

The majority of the experiments outlined in **Task 1** of the approved grant application involving the co-activating effect of AIB1 and  $\Delta$ exon3 AIB1 on EGF signaling in a breast cancer cell model have been completed and have yielded mostly negative results. However, the role of AIB1 and  $\Delta$ exon3 AIB1 in co-activating IGF-1 signaling and its contribution to hormone independence in breast cancer has yet to be determined and may prove to be more interesting. AIB1 knockout studies in mice have decreased levels of serum IGF-1 and display defects in somatic growth, mammary gland development, female reproductive growth, and dwarfism [22, 23]. The role of AIB1 and  $\Delta$ exon3 AIB1 in co-activating an IGF-1 responsive promoter will be examined. The effect of IGF-1 signaling on AIB1 message levels in MCF-7 cells will also be examined. The role of AIB1 and  $\Delta$ exon3 AIB1 in the regulation endogenous gene that is regulated by IGF-1 or important in IGF-1 signaling pathway will be determined.

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